

Practical concepts in Microbiology

Lecture 4

Key terms used in Microbiology

- Sterilization

- Sterilization is a technique used in Microbiology. It is the process of removing or destroying all microorganisms and viruses on or in a **product** is called **sterilization**.

- How the Sterilization can be achieved?

- These procedures include:

Removing microbes by filtration, or destroying them using heat, certain chemicals, or irradiation.

Sterilization and Disinfection

- Destruction of microorganisms means they cannot be “revived” to multiply even when transferred from the sterilized product to an ideal growth medium.

A **sterile** item is one that is absolutely free of microbes, including endospores and viruses.

Disinfection is the process that eliminates most or all pathogens on or in a material.

Unlike sterilization, disinfection suggests that some viable microbes may persist.

Disinfection and Antiseptic

- In practice, the term *disinfection* generally implies the use of antimicrobial chemicals.
- Those used for disinfecting inanimate objects are called **disinfectants**.
- Disinfectants are **biocides** (*bio* means “life,” and *cida* means “to kill”).

When disinfectants are formulated for use on skin, they are called **antiseptics**.

Antiseptics are routinely used to decrease the number of bacteria on skin to prepare for invasive procedures such as surgery.

Decontamination, Pasteurization & Sanitization

- **Decontamination** is a treatment used to reduce the number of pathogens to a level considered safe to handle. The treatment can be as simple as thorough washing, or it may involve the use of heat or disinfectants.
- **Pasteurization** uses a brief heat treatment to reduce the number of spoilage organisms and kill pathogens. Foods and inanimate objects can be pasteurized.
- **Sanitized** generally implies a substantially reduced microbial population that meets accepted health standards. Most people also expect a sanitized object to be clean in appearance. Note that this term does not denote any specific level of control.

Preservation

- **Preservation** is the process of delaying spoilage of foods or other perishable products.
- This is done by adding:
 - Growth inhibiting ingredients or
 - Adjusting storage conditions to impede growth of microorganisms.

Situations that Warrant Different Levels of Microbial Control



- (a) Daily home life;
- (b) foods and food production facilities;
- (c) hospitals;
- (a) water treatment facilities
- (b) other industries.

FIGURE 5.1 Situations that Warrant Different Levels of Microbial Control (a) Daily home life; (b) foods and food production facilities; (c) hospitals; (d) water treatment facilities; (e) other industries.

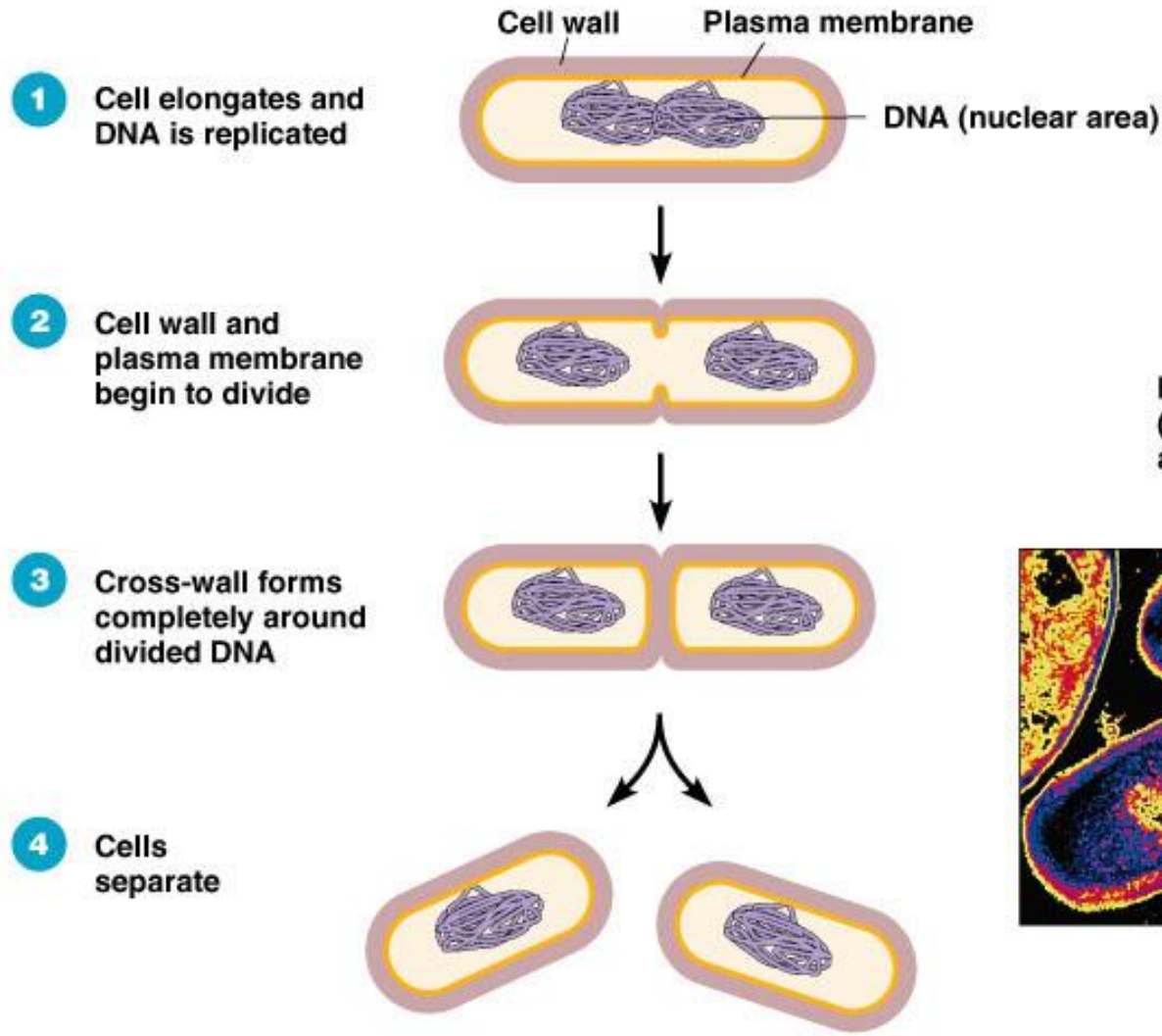
Aseptic techniques

- **Aseptic technique** is a process or procedure used to achieve **asepsis** to prevent the transfer of potentially pathogenic micro-organisms to a susceptible site that may result in the development of infection.
- **Aseptic technique** refers to a set of routine procedures done to prevent sterile solutions and cultures from becoming contaminated by unwanted microorganisms in the **laboratory**.
- **Aseptic** preparation may involve: disinfecting a patient's skin using antiseptic wipes. sterilizing equipment and instruments before a **procedure**. keeping sterilized instruments inside plastic wrappers to prevent contamination before use.

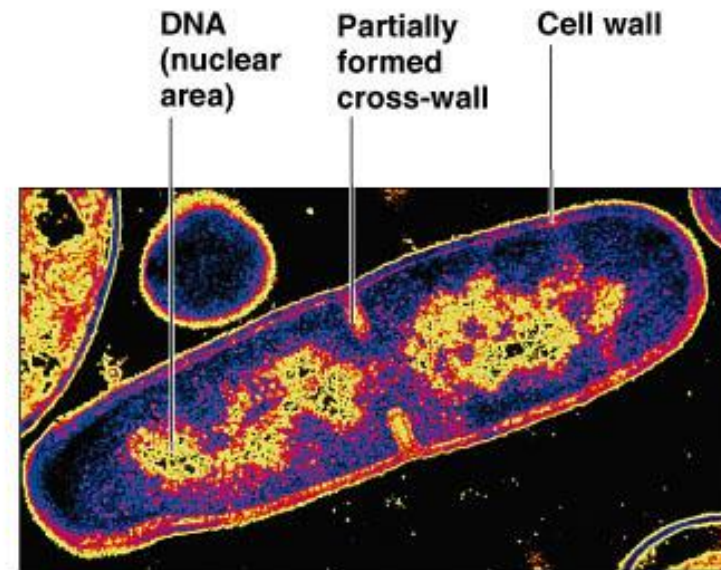
Useful links

- <https://www.youtube.com/watch?v=bRadiLXkqoU>
- <https://www.youtube.com/watch?v=cneascR3OEc>

Binary Fission



(a) A diagram of the sequence of cell division.



(b) A thin section of a cell of *Bacillus licheniformis* starting to divide.

Growth rate in bacteria

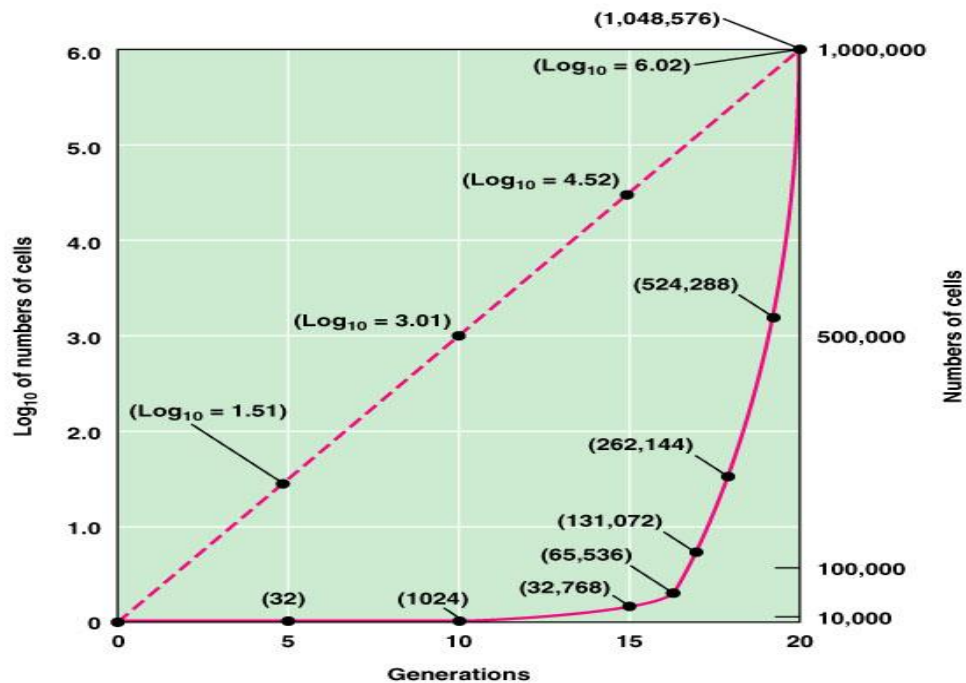


TABLE 4.1 Example of Exponential Growth

Time in Minutes (<i>t</i>)	Initial Population (N_0)	Number of Generations (<i>n</i>)	2^n	Population $N_0 \times 2^n$
0	10	0		10
20	10	1	$2^1 (=2)$	20
40	10	2	$2^2 (=2 \times 2)$	40
60 (1 hour)	10	3	$2^3 (=2 \times 2 \times 2)$	80
80	10	4	$2^4 (=2 \times 2 \times 2 \times 2)$	160
100	10	5	$2^5 (=2 \times 2 \times 2 \times 2 \times 2)$	320
120 (2 hours)	10	6	2^6	640
140	10	7	2^7	1,280
160	10	8	2^8	2,560
180 (3 hours)	10	9	2^9	5,120
200	10	10	2^{10}	10,240
220	10	11	2^{11}	20,480
240 (4 hours)	10	12	2^{12}	40,960

Cell division in bacteria

- So here we have seen
- How the bacteria divide?
 - Binary fission
 - 20-min-2 days
 - What is a colony?
 - Colony is a visible mass of cell arising from a parent cell.
 - How to culture the bacteria?
 - Streaking on the culture medium (Nutrient Agar medium)
 - Mixed culture (different types of colonies/different types of bacteria)

Growth media

- Types of media

- Solid media Agar
- Liquid media does not have agar
- Semisolid media  Agar in small quantity

- Types of cultures

- Mixed culture: different types of bacteria cells
- Pure culture: Single type of bacteria
- Useful link: <https://www.youtube.com/watch?v=EjnQ3peWRek>

Measurement of Bacterial cell numbers

- **Viable cell count methods**

- Plate count
- Membrane filtration
- Most probable number

- **Why do we use viable cell count methods?**

- Viable cell counts are invaluable for monitoring bacterial growth in samples such as food and water that often contain numbers too low to be seen using a direct microscopic count.

- **Limitations of using viable cell count methods**

- Viable cell counts are used to quantify the number of cells capable of multiplying.
- These methods require knowledge of appropriate growth conditions for a particular microorganism as well as the time to allow growth to occur.
- By using selective and differential media, a particular species of bacteria can often be enumerated.

Measurement of Cell Numbers

Viable cell count

Viable Count: A viable cell is defined as a cell which is able to divide and increase cell numbers. **Plate counts** measure the number of viable cells in a sample by exploiting the fact that an isolated cell on a nutrient agar plate will give rise to one colony. A simple count of the colonies determines how many cells were in the initial sample

Here it is assumed that each viable cell will form one colony. Therefore, viable count is often called plate count or colony count. The ideal number of colonies to count is between 30 and 300 samples frequently contain many more bacteria than this, it is usually necessary to dilute the samples before plating the cells.

Samples are normally diluted in 10-fold increments, making the resulting math relatively simple. The **diluent**, or sterile solution used to make the dilutions, is generally physiological saline (0.85% NaCl in water).

Measurement of Cell Numbers

Membrane filtration technique

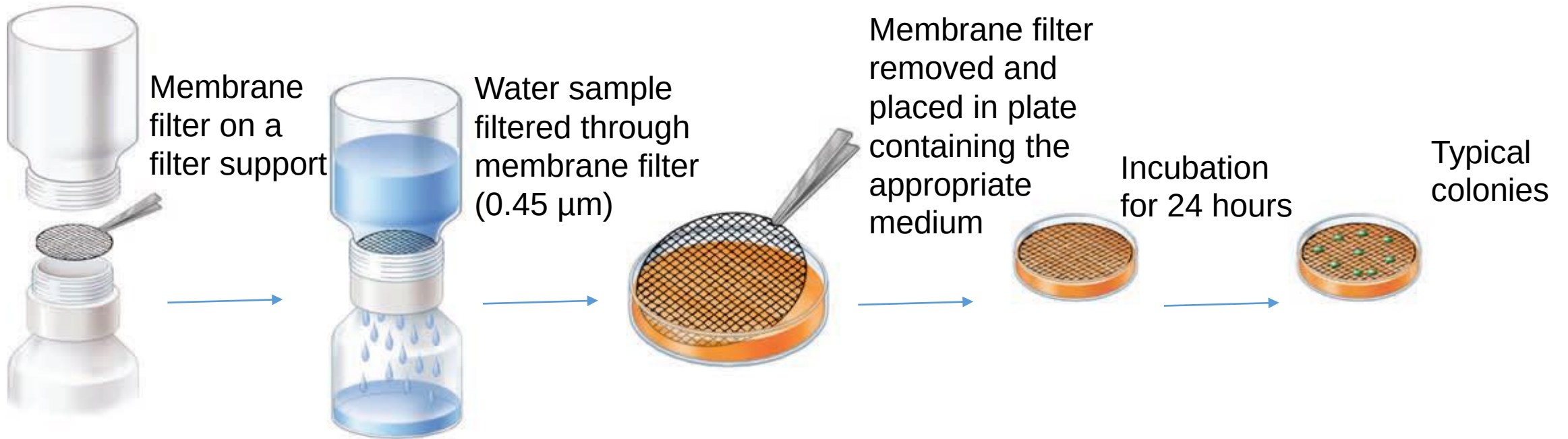
Filtration: Bacteria can be counted this way when it is small enough in lakes or pure streams. This occurs through the bacteria being filtered out and retained on the filter surface, which then gets transferred to a petri dish of nutrient medium where colonies will appear over the surface.

Advantages and limitations of viable or plating technique

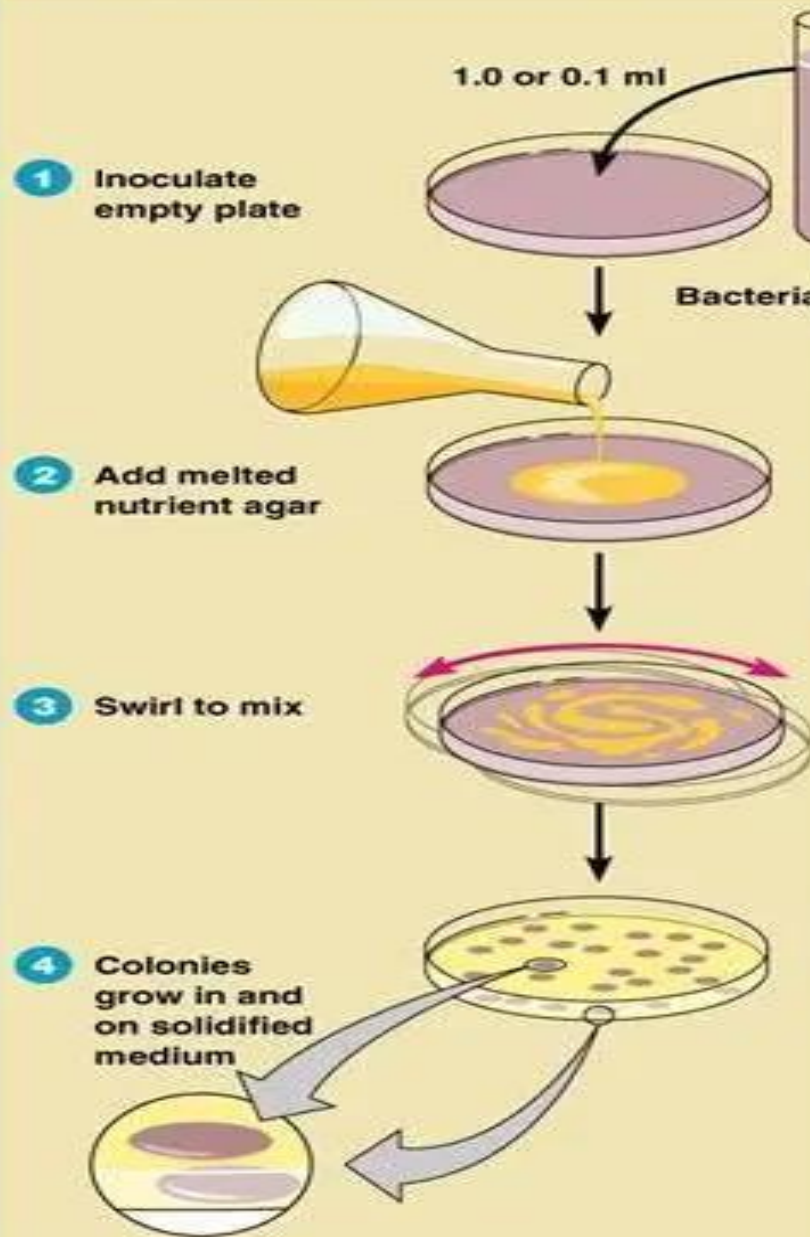
- Plating techniques are simple, sensitive, and widely used for viable counts of bacteria and other microorganisms in samples of food, water, and soil. Several problems, however, can lead to inaccurate counts.
- Low counts will result if clumps of cells are not broken up and the microorganisms well dispersed.
- Because it is not possible to be absolutely certain that each colony arose from an individual cell, the results are often expressed in terms of **colony forming units (CFU)** rather than the number of microorganisms.
- The samples should yield between 30 and 300 colonies for most accurate counting.
- The counts will also be low if the agar medium employed cannot support growth of all the viable microorganisms present.
- The hot agar used in the pour-plate technique may injure or kill sensitive cells; thus spread plates sometimes give higher counts than pour plates.

The Membrane Filtration Procedure. Membranes with different pore sizes are used to trap different microorganisms. Incubation times for membranes also vary with the medium and microorganism.

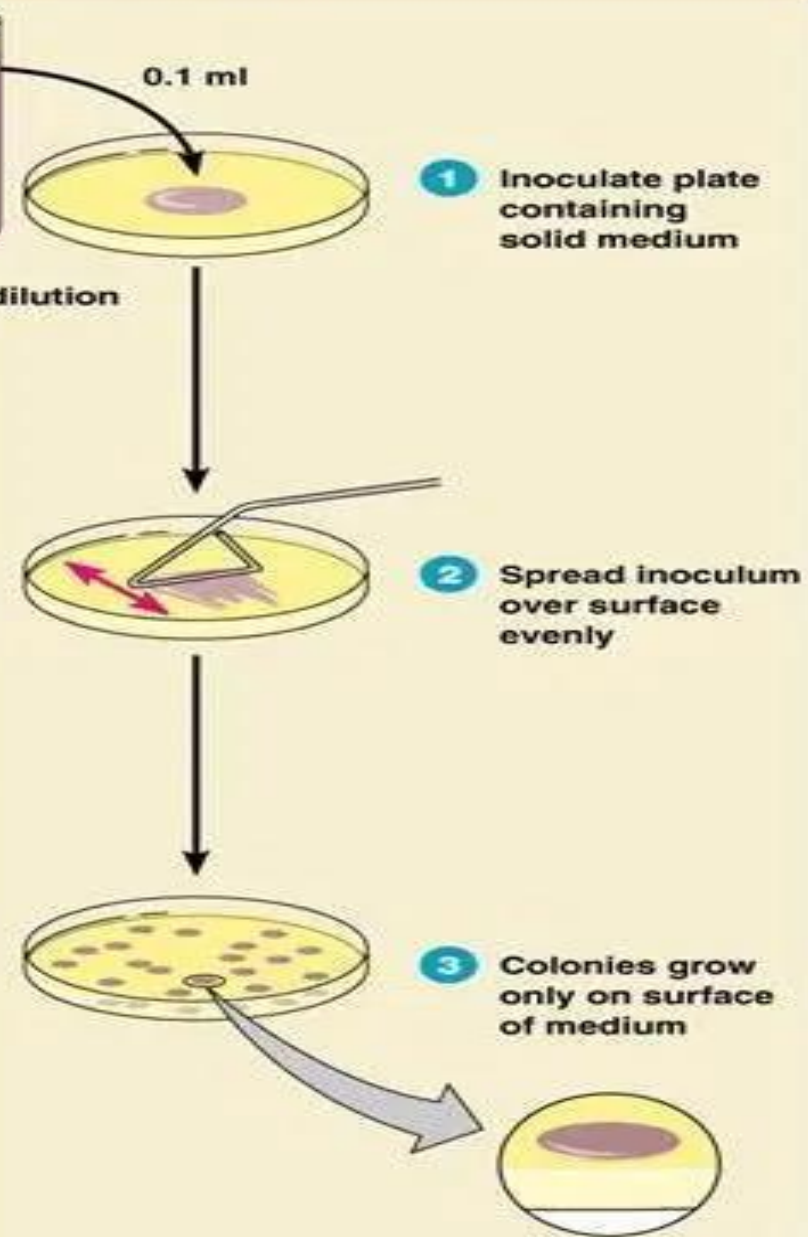
A known volume of liquid is passed through a sterile membrane filter, which has a pore size that retains bacteria. The filter is subsequently placed on an appropriate agar medium and then incubated. The number of colonies that grow on the filter indicates the number of bacteria in the volume filtered.



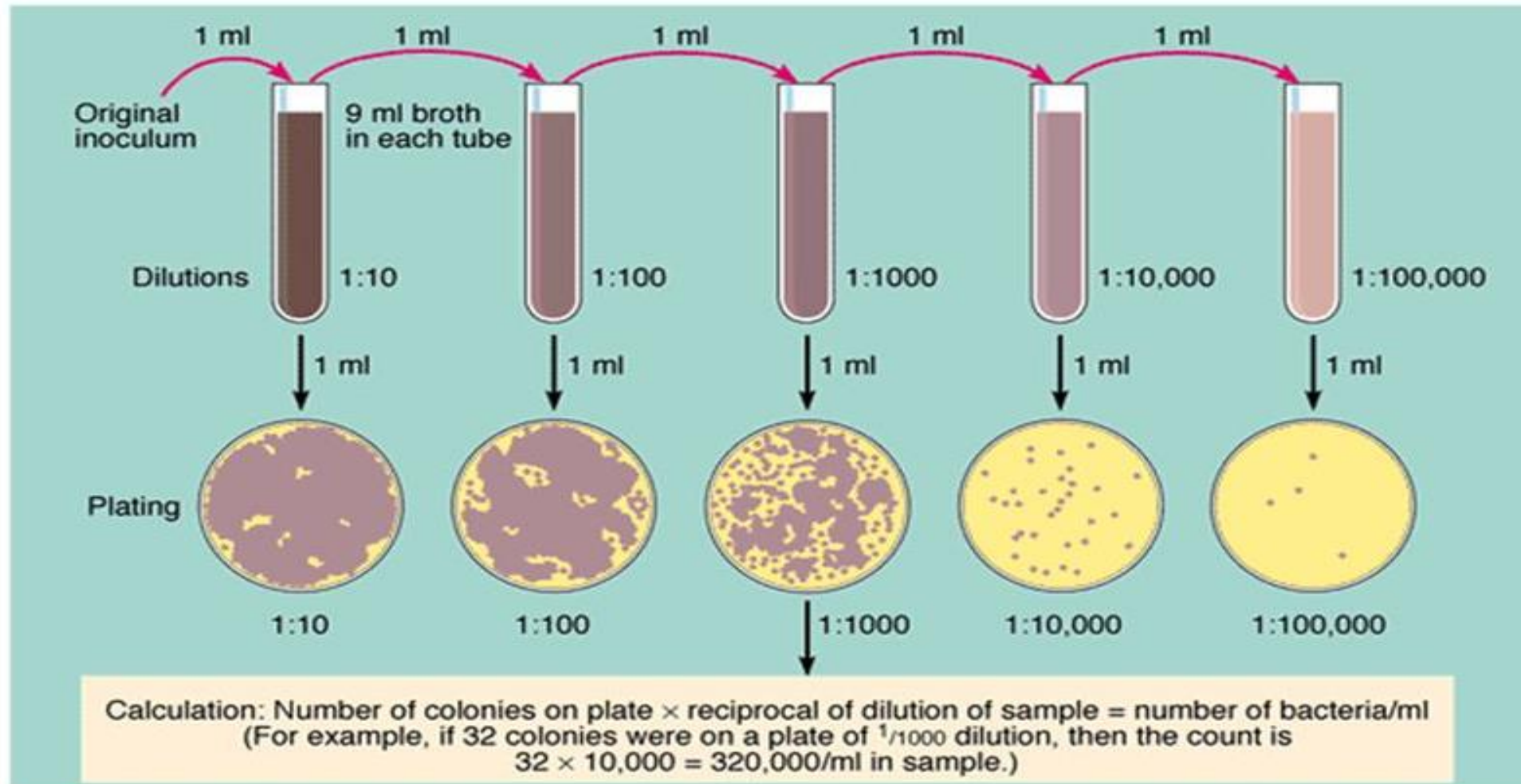
(a) The pour plate method



(b) The spread plate method



Serial Dilutions are Used with the Plate Count Method to Measure Numbers of Bacteria



$$\frac{\text{volume of sample}}{\text{volume of sample} + \text{volume of diluent}}$$

For a ten-fold dilution, 1 ml of sample is added to 9 ml of diluent. In this case, the dilution factor for that test tube will be: 1:10

$$\text{Dilution factor} = \frac{1 \text{ ml}}{1 \text{ ml} + 9 \text{ ml}} = \frac{1}{10} = 10^{-1}$$

Think about:
why do we make dilutions?
How can we calculate CFU/ml?

